Amendments to the Specification

Please replace the paragraph starting with Fig. 1 on page 8 with the following amended paragraph:

FIG. 1 is an elution pattern of a saccharide, obtained by α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus* C9 strain, when determined on high-performance liquid chromatography.

Please replace the paragraph starting with Fig. 2 on page 8 with the following amended paragraph:

FIG. 2 is a nuclear resonance spectrum (^{1}H-NMR) of cyclotetrasaccharide, obtained by the enzymatic reaction using α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 3 on page 9 with the following amended paragraph:

FIG. 3 is a nuclear resonance spectrum (^{13}C-NMR) of cyclotetrasaccharide, obtained by the enzymatic reaction using α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 5 on page 9 with the following amended paragraph:

FIG. 5 shows the thermal influence on the enzymatic activity of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 6 on page 9 with the following amended paragraph:

FIG. 6 shows the pH influence on the enzymatic activity of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 7 on page 9 with the following amended paragraph:

FIG. 7 shows the thermal stability of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 8 on page 9 with the following amended paragraph:

FIG. 8 shows the pH stability of α-isomaltosylglucosaccharide-forming enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 9 on page 9 with the following amended paragraph:

FIG. 9 shows the thermal influence on the enzymatic activity of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 10 on page 9 with the following amended paragraph:

FIG. 10 shows the pH influence on the enzymatic activity of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 11 on page 9 with the following amended paragraph:

FIG. 11 shows the thermal stability of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globisporus* C9 strain.

Please replace the paragraph starting with Fig. 12 on page 10 with the following amended paragraph:

FIG. 12 shows the pH stability of α-isomaltosyl-transferring enzyme from a microorganism of the species *Bacillus globiformis* C9 strain.

Please replace the paragraph starting with Fig. 50 on page 14 with the following amended paragraph:

FIG. 50 is an x-ray diffraction spectrum for an anhydrous crystalline powder of the cyclotetrasaccharide of the present invention, obtained by drying *in vacuo* at 40°C, when determined on x-ray powder diffraction analysis.

Please replace the page 44 with the following amended page:

as a coffee, cocoa, juice, carbonated beverage, sour milk beverage, and beverage containing a lactic acid bacterium; instant food products such as instant pudding mix, instant hot

cake mix, instant juice or soft drink, instant coffee, "*sokuseki-shiruko*" (an instant mix of adzuki-bean soup with rice cake), and instant soup mix; and other foods and beverages such as solid foods for babies, foods for therapy, health/tonic drinks, peptide foods, and frozen foods. The cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be arbitrarily used to prolong or retain the flavor and taste of fresh-baked Japanese and Western confectioneries and to improve the taste preference of feeds and pet foods for animals and pets such as domestic animals, poultry, honey bees, silk worms, and fish; and also they can be arbitrarily used as a sweetener, taste-improving agent, flavoring substance, quality-improving agent, and stabilizer in other products in a paste or liquid form such as a tobacco, cigarette, tooth paste, lipstick, rouge, lip cream, internal liquid medicine, tablet, troche, cod liver oil in the form of drop, cachou, oral refrigerant, gargle, cosmetic, and pharmaceutical. When used as a quality-improving agent or stabilizer, the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be arbitrarily used in biologically active substances susceptible to lose their effective ingredients and activities, as well as in health foods and pharmaceuticals containing the biologically active substances. Examples of such biologically active substances are liquid preparations containing lymphokines such as α-, β- and γ-interferons, tumor necrosis factor-α (TNF-

Please replace the page 45 with the following amended page:

α), tumor necrosis factor-β (TNF-β), macrophage migration inhibitory factor, colony-stimulating factor, transfer factor, and interleukin 2; liquid preparations containing hormones such as insulin, growth hormone, prolactin, erythropoietin, and follicle-stimulating hormone; biological preparations such as BCG vaccine, Japanese encephalitis vaccine, measles vaccine, live polio vaccine, smallpox vaccine, tetanus toxoid, Trimeresurus antitoxin, and human immunoglobulin; antibiotics such as penicillin, erythromycin, chloramphenicol, tetracycline, streptomycin, and kanamycin sulfate; liquid preparations containing vitamins such as thiamine, riboflavin, L-ascorbic acid, cod liver oil, carotenoid, ergosterol, and tocopherol; highly unsaturated fatty acids and ester derivatives thereof such as EPA, DHA, and arachidonic acid; solutions of enzymes such as lipase, elastase, urokinase, protease, β-amylase, isoamylase, glucanase, and lactase; extracts such as ginseng extract, snapping turtle extract, chlorella extract, aloe extract, and propolis extract; and royal jelly. By using the cyclotetrasaccharide and the saccharide compositions comprising

the same of the present invention, the above biologically active substances and other pastes of living microorganisms such as viruses, lactic acid bacteria, and yeasts can be arbitrarily prepared into health foods and pharmaceuticals in a liquid, paste, or solid form, which have a satisfactorily-high stability and quality with less fear of losing or inactivating their effective ingredients and activities.

As mentioned above, the following effects and features are also effectively exerted when used with other ingredients which are generally used externally: The effects of preventing

Please replace the paragraph starting with "Similary" starting on page 46 with the following amended paragraph:

Similarly as other naturally occurring saccharides, since the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention quite scarcely stimulate the skin when applied thereupon and effectively retain the moisture in the skin, they can be advantageously incorporated into external dermal compositions for use. In the external dermal compositions, the cyclotetrasaccharide and the saccharide compositions comprising the same of the present invention can be usually used in an appropriate combination with one or more dermatologically applicable other ingredients of oils and lipids, waxes, hydrocarbons, fatty acids, esters, alcohols, surfactants, dyes, flavors, hormones, vitamins, plant extracts, animal extracts, microbial extracts, salts, ultraviolet absorbents, photosensitizing dyes, antioxidants, antiseptics/bactericides, antiperspirants/deodorants, refreshments, chelating agents, skin whitening agents, anti-inflammatories, enzymes, saccharides, amino acids, and thickening agents. For example, in the field of cosmetics, the external dermal compositions can be provided in the form of a lotion, cream, milky lotion, gel, powder, paste, or block, for example, cleaning cosmetics such as soaps, cosmetic soaps, washing powders for the skin, face washing creams, facial rinses, body shampoos, body rinses, shampoos, and powders for washing hair; cosmetics for hair such as set lotions, hair blows, stick pomades, hair creams, pomades, hair sprays, hair liquids, hair tonics, hair lotions, hair restorers, hair dyes, treatments for scalp, hair cosmetics, gloss-imparting hair oils, hair oils, and combing oils; base cosmetics such as cosmetic lotions, vanishing creams, emollient creams, emollient lotions, cosmetic packs in the form of a jelly peel off, jelly wiping, paste washing, powders, cleansing creams, cold creams, hand creams, hand lotions, milky lotions, moisture-imparting liquids, after/before shaving lotions, after shaving creams, after

shaving foams, before shaving creams, and baby oils; makeup cosmetics such as foundations in the form of a liquid, cream or solid, talcum powders, baby powders, body powders, perfume powders, makeup bases, powders in the form of a cream, paste, liquid, solid or powder, eye shadows, eye creams, mascaras, eyebrow pencils, eyelash makeups, rouges, rouge lotions; perfume cosmetics such as perfumes, paste/powder perfumes, *eau de Colognes*, perfume *Colognes*, and *eau de toilette*; suntan and suntan preventive cosmetics such as suntan creams, suntan lotions, and suntan oils; nail cosmetics such as manicures, pedicures, nail colors, nail lacquers, and nail makeup materials; eyeliner cosmetics; rouges and lipsticks such as lipsticks, lipcreams, paste rouges, and lip-glosses; oral cosmetics such as tooth pastes and mouth washes; and bath cosmetics such as bath salts/oils, and bath cosmetic materials. In the field of pharmaceuticals, the external dermal compositions can be provided in the form of a wet compresses, sprays, applications, bath agents, sticking agents, ointments, pastes, embrocations, lotions, and cataplasms.

Please replace page 49 with the following amended page:

alcohol, cetanol, setostearyl alcohol, stearyl alcohol, oleyl alcohol, behenyl alcohol, lanolin alcohol, hydrogenated lanolin alcohol, hexyldecanol, octyldodecanol, and polyethylene glycol; lower alcohols including polyalcohols such as ethanol, propanol, isopropanol, butanol, ethylene glycol, propylene glycol, and glycerine; and derivatives thereof.

Examples of the esters usable in the present invention are hexyl laurate, isopropyl myristate, myristyl myristate, cetyl myristate, octyl dodecyl myristate, isopropyl palmitate, butyl stearate, cholesteryl stearate, cholesteryl acetate, cholesteryl n-lactate, cholesteryl caproate, cholesteryl laurate, cholesteryl myristate, cholesteryl palmitate, cholesteryl stearate, cholesteryl 12-hydroxystearate, decyl oleate, octyldodecyl oleate, isopropyl lanolin fatty acid, glycerine trimyristate, propylene glycol dioleate, myristyl lactate, cetyl lactate, lanolin acetate, hexyldecyl dimethyloctanoate, and derivatives thereof.

The surfactants usable in the present invention are, for example, anionic surfactants such as zinc laurate, zinc myristate, zinc palmitate, magnesium stearate, sodium lauryl sulfate, sodium polyoxyethylene laurylether sulfate, triethanolamine polyoxyethylene laurylether sulfate, polyoxyethylene cetylether phosphate, polyoxyethylene alkylphenylether phosphate, sodium N-lauroyl sarcosinate, coconut fatty acid sarcosinate triethanolamine, coconut fatty acid sodium methyltaurate, and soybean phospholipid; cationic

surfactants such as stearyltrimethylammonium chloride, distearyldimethylammonium chloride, benzalkonium chloride, cetylpyridinium chloride, alkylisoquinolinium bromide, and

Please replace the first paragraph on page 51 with the following amended paragraph:

The fragrances used generally in external dermal uses can be roughly classified into natural plant and animal fragrances, synthetic fragrances, and mixtures thereof in an appropriate combination. Examples of the animal fragrances include musk, civetone, and ambergris. The plant fragrances are, for example, distillations, i.e., essential oils, obtainable by distilling, for example, with water vapor anise seeds, basil leaves, caraway fruit, cinnamon barks, coriander seeds, lavender flowers, nutmeg seeds, peppermint leaves, rose flowers, rosemary flowers, seeds, and leaves, and thyme leaves; extracts classified generally into absolutes, resinoids, oleo resins, and tinctures depending on properties and processes. Examples of the synthetic fragrances are acetophenone, anisole, benzyl alcohol, butyl acetate, camphor, citral, citronellol, cuminaldehyde, estragol, ethylvaniline, geranyl acetate, linarol, menthol, methyl p-cresol, methyl salicylate, phenyl acetate, vanillin, and derivatives thereof. In the present invention, fragrance compositions mixed with the aforesaid flavors in an appropriate combination can be arbitrarily used.

Please replace the first paragraph on page 52 with the following amended paragraph:

Examples of the plant extracts usable in the present invention are, in addition to the aforesaid plant extracts used as fragrances, extracts such as those of chamomile, sage, aloe, scarlet sage, *Angelica keiskei*, avocado, nettle, fennel, oolong tea, coak tree bark, barley, *Abelmoschus esculentus*, allspice, seaweed, chinese quince, licorice, quince seed, gardenia, *Sasa albo-marginata*, cinnamon, black tea, rice bran, fermented rice bran, *Stevia rebaudiana*, celery, Japanese green gentian, soy bean, thyme, tea, common camellia, *Ligusticum acutilobum*, corn, carrot, *Rosa rugosa*, *hinoki* (Japanese cypress), dishcloth gourd, safflower, pine, peach, eucalyptus, creeping saxifrage, *yuzu* (citron), lily, Job's tears, Mugwort, *Cyanophta* (blue-green algae), seaweed, apple, *Serratia marcescens*, and lettuce; and compounds isolated from plants such as hinokitiol, azulene, chlorophyll, and glycyrrhizin. The animal extracts usable in the present invention include placenta extracts.

Please replace the first paragraph on page 54 with the following amended paragraph:

Examples of the aseptics and bactericides usable in the present invention include, in addition to the aforesaid compounds with aseptic or bactericidal activities, phenol compounds such as phenol, p-chloro metacresol, resorcin, p-oxy benzoate, and cresol; acid compounds including those in a salt form such as benzoic acid, sorbic acid, salicylic acid, and boric acid; bisphenol halides such as hexachlorophene, bithionol, and dichlorophene; amides such as 3,4,4'-trichlorocarvaniride, undecylenic acid monoethanolamide; quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, and decalinium chloride; chlorhexidine hydrochloride, 1-hydroxypyridine-2-thione, lysozyme chloride; and derivatives thereof.

Please replace the first paragraph on page 71 with the following amended paragraph:

As evident from the results in Table 3, the enzyme activity was greatly inhibited by Hg^{2+}, Cu^{2+}, and EDTA, and was also inhibited by Ba^{2+} and Sr^{2+}. It was also found that the enzyme was activated by Ca^{2+} and Mn^{2+}.

Please replace the first paragraph on page 73 with the following amended paragraph:

As evident from the results in Table 4, the enzyme activity was greatly inhibited by Hg^{2+} and was also inhibited by Cu^{2+}. It was also found that the enzyme was not activated by Ca^{2+} and not inhibited by EDTA.

Please replace the first paragraph on page 79 with the following amended paragraph:

A faction of α-isomaltosyl-transferring enzyme, which had been separated from a fraction with α-isomaltosylglucosaccharide-forming enzyme by the affinity chromatography in Experiment 7-1, was dialyzed against 10 mM phosphate buffer (pH 7.0) containing 1 M ammonium sulfate. The dialyzed solution was centrifuged to remove insoluble impurities, and the resulting supernatant was fed to hydrophobic chromatography using 350 ml of "BUTYL-TOYOPEARL 650 M", a gel commercialized by Tosoh Corporation, Tokyo, Japan. The enzyme adsorbed on the gel and then it was eluted at about 0.3 M ammonium sulfate when eluted with a linear gradient decreasing from 1 M to 0 M of ammonium sulfate, followed by collecting fractions with the enzyme activity. The

fractions were pooled and dialyzed against 10 mM phosphate buffer (pH 7.0) containing 1 M ammonium sulfate. The resulting dialyzed solution was centrifuged to remove impurities and fed to affinity chromatography using "SEPHACRYL HR S-200" gel to purify the enzyme. The amount of enzyme activity, specific activity, and yield of the α-isomaltosyl-transferring enzyme in each purification step are in Table 6.

Please replace the first line of the second paragraph on page 79 with the following amended line:

"A fraction of α-isomaltosyl-transferring enzyme,"

Please replace the first line of the first paragraph on page 83 with the following amended line:

"As evident from the results in Table 7, the enzyme"

Please replace the first line of the second paragraph on page 85 with the following amended line:

"As evident from the results in Table 8, the enzyme"

Please replace Table 9 on page 87 with the following amended table:

Table 9

Peptide name	Internal partial amino acid sequence
P64	aspartic acid-alanine-serine-alanine-asparagine-valine-threonine-threonine
P88	tryptophan-serine-leucine-glycine-phenylalanine-methionine-asparagine-phenylalanine
P99	asparagine-tyrosine-threonine-aspartic acid-alanine-tryptophan-methionine-phenylalanine

Please replace Table 10 on page 88 with the following amended table:

Table 10

Peptide name	Internal partial amino acid sequence
P22	glycine-asparagine-glutamic acid-methionine-arginine-asparagine-glutamine-tyrosine
P63	isoleucine-threonine-threonine-tryptophan-proline-isoleucine-glutamic acid-serine

(Continued)

Peptide name	Internal partial amino acid sequence
P71	tryptophan-alanine-phenylalanine-glycine-leucine-tryptophane-methionine-serine

Please replace the first line of the first paragraph on page 99 with the following amended line:

"As evident from the results in Table 13, the enzyme"

Please replace the first line of the second paragraph with the following amended line:

As evident from the results in Table 14, the enzyme"

Please replace Table 16 on page 105 with the following amended table:

Table 16

Peptide name	Internal partial amino acid sequence
PN21	asparagine-tryptophan-tryptophan-methionine-serine-lysine
PN38	threonine-aspartic acid-glycine-glycine-glutamic acid-methionine-valine-tryptophane
PN69	asparagine-isoleucine-tyrosine-leucine-proline-glutamine-glycine-aspartic acid

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Please replace the first line of the first paragraph on page 114 with the following amended line:

"As evident from the results in Table 19, it was"

Please replace the last line on page 120 with the following amended line:

"As evident from the results in Table 21, it was"

Please replace the fourth line on page 127 with the following amended line:

"glucosylmaltose or panose were mainly formed from maltose as a"

Please replace the fifth line of the first paragraph on page 128 with the following amended line:

"residue binds via the α-linkage to OH-6 of glucose at the non-"

Please replace the third line on page 132 with the following amended line:

"invention had the reducing power. To a 1%"

Please replace the line 14 on page 132 with the following amended line:

"measured for reducing power after keeping the sampled solutions"

Please replace lines 2 and 3 of the second paragraph on page 133 with the following amended lines:

"formation enzyme of the present invention has the ability to form dextran, it was tested in accordance with the method in"

Please replace line 14 of the second paragraph on page 134 with the following amended lines:

"and stirred for washing. Each resulting solution was"

Please replace line 5 of the first paragraph on page 135 with the following amended lines:

"activity because it did not form dextran when it acted on"

Please replace the second paragraph on page 141 with the following amended paragraph:

The test on the formation of cyclotetrasaccharide by the α-isomaltosylglucosaccharide-forming enzyme and α-isomaltosyl-transferring enzyme was conducted using saccharides. For the test, prepared a solution of maltose, maltotriose, maltotetraose, maltopentaose, amylose, soluble starch, "PINE-DEX #100" (a partial starch hydrolyzate commercialized by Matsutani Chemical Ind., Tokyo, Japan), or glycogen from oyster commercialized by Wako Pure Chemical Industries Ltd., Tokyo, Japan was prepared.

Please replace number (1) on page 144 with the following amended paragraph:

(1) The α-isomaltosylglucosaccharide-forming enzyme of the present invention acts on a glucose residue at the non-reducing end of an α-1,4 glucan chain of glycogen and partial starch hydrolyzates, etc., and intermolecularly transfers the glucose residue to OH-6 of a glucose residue at the non-reducing end of another α-1,4 glucan chain of glycogen to form an α-1,4 glucan chain having an α-isomaltosyl residue at the non-reducing end;

Please replace the last line on page 146 with the following amended line:

"cyclotetrasaccharide on HPLC. The results are in Table 31."

Please replace the first line of the last paragraph on page 148 with the following amended line:

"As is evident from the results in Table 32, the formation"

Please replace the last line of the second paragraph on page 152 with the following amended line:

"change into an anhydrous crystal after being heated up to 250°C."

Please replace line 22 on page 154 with the following amended line:

"amorphous and anhydrous forms when dried *in vacuo*."

Please replace the first paragraph on page 155 with the following amended paragraph:

To study the saturation concentration of cyclotetrasaccharide in water at 10-90_C, 10 ml of water was placed in a glass vessel with a seal cap, and then mixed with cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, in an excessive amount over a level dissolving completely at respective temperatures, cap-sealed, and stirred for two days while keeping at respective temperatures of 10-90_C until being saturated. Each resulting saturated solution of cyclotetrasaccharide was membrane filtered to remove undissolved cyclotetrasaccharide, and each filtrate was then examined for moisture content by the drying loss method to determine a saturation concentration of cyclotetrasaccharide at respective temperatures. The results are in Table 34.

Please replace the first paragraph on page 159 with the following amended paragraph:

A crystalline cyclotetrasaccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, and a commercialized polypeptone, Nihonseiyaku K.K., Tokyo, Japan, were dissolved in deionized water to obtain a 10% (w/v) cyclotetrasaccharide solution containing 5% (w/v) polypeptone. Four milliliters of the resulting solution were placed in a glass test tube, sealed, and heated at 100_C for 30 to 90 min. After allowing to stand for cooling at ambient temperature, each of the resulting solutions was measured for coloration degree to examine on their amino carbonyl reactivity. In parallel, as a control, a solution with only polypeptone was provided and similarly treated as above. The coloration degree was evaluated based on the level of the absorbance, measured in a cell with 1-cm light pass at a wavelength of 480 nm, minus the control. The results are in Table 38.

Please replace the second paragraph on page 165 with the following amended paragraph:

The acute toxicity of a crystalline cyclotetra-saccharide, penta- or hexa-hydrate, obtained by the method in Experiment 30, was tested by orally administering it to mice. As a result, it was revealed that cyclotetrasaccharide had relatively low

toxicity and did not induce death of mice even when administered at a highest possible dose. Based on this, the LD_{50} of cyclotetrasaccharide was at least 50 g/kg mouse body weight, though the data were not so accurate.

Please replace line 8 of the second paragraph on page 179 with the following amended line:

"ASPARTAME" (L-aspartyl-L-phenylalanine methyl ester), and the

Please replace the second paragraph on page 184 with the following amended paragraph:

Ten parts by weight of beans as a material in a usual manner were boiled in a usual manner after the addition of water, removed the astringency, lye, and water-soluble impurities to obtain about 21 parts by weight of raw bean jam in the form of a granule. To the raw bean jam were added 14 parts by weight of sucrose, five parts by weight of a syrup containing cyclotetrasaccharide, obtained by the method in Example A-3, and four parts by weight of water, and the resulting mixture was boiled, admixed with a small amount of salad oil, and then kneaded up without pasting the beans to obtain about 35 parts by weight of the desired product, *an*. Since the product has a satisfactory stability, mouth feel, taste, and flavor, and does not substantially exhibit syneresis and excessive color upon baking, it can be arbitrarily used as a material for confectioneries such as a bean jam bun, "*manju*" (a kind of Japanese confectionery with bean jam), bean-jam-filled wafer, and ice cream/candy.

Please replace the last paragraph on page 187 with the following amended paragraph:

The product is a high quality bath salt enriched with *yuzu* flavor and used by diluting in hot water by 100-10,000 folds, and it moisturizes and smooths the skin and does not make one feel cold after taking a bath therewith.

Please replace the paragraph on page 192 with the following amended paragraph:

cyclotetrasaccharide having the structure of cyclo{66)-α-D-glucopyranosyl-(163)-α-D-glucopyranosyl-(166)-α-D-glucopyranosyl-(163)-α-D-glucopyranosyl-(16} or a composition comprising the same can be produced on an industrial scale and at a relatively low cost. Since these cyclotetrasaccharides and the saccharide comprising the same have substantially no or low

reducibility, substantially do not cause the amino carbonyl reaction, substantially do not exhibit hygroscopicity, have easily handleability, have mild sweetness, adequate viscosity, moisture-retaining ability, inclusion ability, and substantially no digestibility, they can be advantageously used in a variety of compositions such as food products, cosmetics, pharmaceuticals as a sweetener, material for low caloric foods, taste-improving agent, flavor-improving ability, quality-improving agent, syneresis-preventing agent, stabilizer, filler, inclusion agent, and base for pulverization. The present invention, having these outstanding functions and effects, is a significantly important invention that greatly contributes to this art.